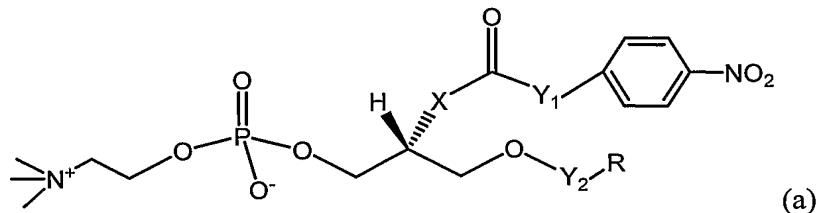


**What is claimed is:**

1. A method for measuring enzymatically active Lipoprotein-associated Phospholipase A2 (Lp-PLA2) in a sample comprising:
  - (a) contacting an immobilized binder, which specifically binds Lp-PLA2, with the sample;
  - (b) washing the immobilized binder to remove an enzymatically active unbound material or an interfering substance(s);
  - (c) contacting the bound Lp-PLA2 with a substrate converted to a detectable product in the presence of Lp-PLA2; and
  - (d) measuring detectable product indicative of enzymatically active Lp-PLA2 in the sample.
2. The method of claim 1, wherein the sample is a serum sample, a plasma sample, an EDTA treated plasma sample or an EDTA treated serum sample.
3. The method of claim 1, wherein the immobilized binder is an antibody.
4. The method of claim 3, wherein the antibody is a monoclonal antibody, a phage display antibody, or a polyclonal antibody.
5. The method of claim 4, wherein the antibody is a monoclonal antibody.
6. The method of claim 1 wherein the enzymatically active unbound material is a phospholipase.
7. The method of claim 1 wherein the interfering substance(s) is a free-thiol compound.
8. The method of claim 1, wherein the substrate is selected from the group consisting of



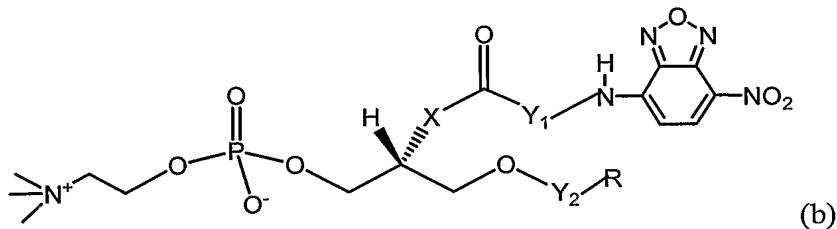
25

wherein,

X is selected from the group consisting of O, S, and -O(CO)-;

R is selected from the group consisting of (CH<sub>2</sub>)<sub>4</sub>CH<sub>3</sub>, (CH<sub>2</sub>)<sub>6</sub>CH<sub>3</sub>, (CH<sub>2</sub>)<sub>8</sub>CH<sub>3</sub>, (CH<sub>2</sub>)<sub>10</sub>CH<sub>3</sub>, (CH<sub>2</sub>)<sub>12</sub>CH<sub>3</sub>, (CH<sub>2</sub>)<sub>14</sub>CH<sub>3</sub>, and (CH<sub>2</sub>)<sub>7</sub>CH=CH(CH<sub>2</sub>)<sub>2</sub>CH<sub>3</sub>;

$Y_1$  is selected from the group consisting of  $(CO)_{1-2}$  and  $(CH_2)_{2-7}$ ; and  
 $Y_2$  is selected from the group consisting of CO and CH<sub>2</sub>;



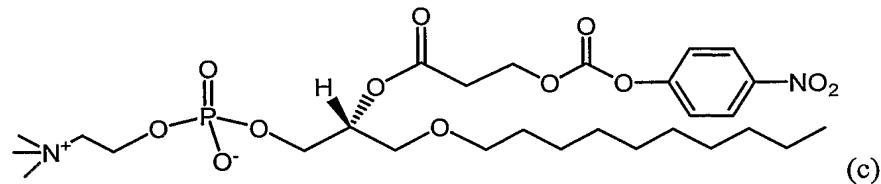
5 wherein,

$X$  is selected from the group consisting of O, S, and -O(CO)-;

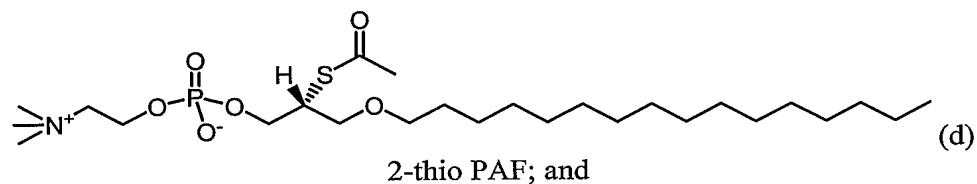
$R$  is selected from the group consisting of  $(CH_2)_{4}CH_3$ ,  $(CH_2)_{6}CH_3$ ,  $(CH_2)_{8}CH_3$ ,  $(CH_2)_{10}CH_3$ ,  $(CH_2)_{12}CH_3$ ,  $(CH_2)_{14}CH_3$  and  $(CH_2)_7CH=CH(CH_2)_2CH_3$ ;

$Y_1$  is selected from the group consisting of  $(CO)_{1-2}$  and  $(CH_2)_{2-7}$ ; and

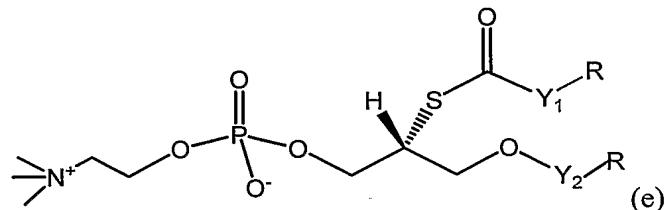
10  $Y_2$  is selected from the group consisting of CO and CH<sub>2</sub>;



15 1-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine (MNP);



2-thio PAF; and



20 wherein

$X$  is selected from the group consisting of O, S, and -O(CO)-;

$R$  is selected from the group consisting of  $(CH_2)_{4}CH_3$ ,  $(CH_2)_{6}CH_3$ ,  $(CH_2)_{8}CH_3$ ,  $(CH_2)_{10}CH_3$ ,  $(CH_2)_{12}CH_3$ ,  $(CH_2)_{14}CH_3$ , and  $(CH_2)_7CH=CH(CH_2)_2CH_3$ ;

$Y_1$  is selected from the group consisting of  $(CO)_{1-2}$  and  $(CH_2)_{2-7}$ ; and

$Y_2$  is selected from the group consisting of CO or  $CH_2$ .

9. The method of claim 8 where in the substrate is an oxidized derivative of (a), (b), (c), (d) or (e).
- 5 10. The method of claim 1, wherein the detectable product has a radioactive, colorimetric, paramagnetic or fluorescent label.
11. The method of claim 1 wherein the detectable product is measured fluorimetrically, colorimetrically, paramagnetically or via radiation.
12. The method of claim 1 which further comprising comparing the measured 10 detectable product of step (d) to detectable product in a control comprising an enzymatically active Lp-PLA2 standard.
13. The method of claim 12 wherein the enzymatically active Lp-PLA2 standard is a recombinant Lp-PLA2 protein or a native Lp-PLA2 protein.
14. The method of claim 13 wherein the recombinant Lp-PLA2 protein is expressed 15 in a baculovirus expression system or a mammalian expression system.
15. The method of claim 1 wherein the immobilized binder is bound to a multi-well plate, a magnetic bead, or a latex bead.
16. The method of claim 12 wherein a difference in detectable product in the sample compared to the standard is due to a difference in Lp-PLA2 activity in the 20 sample compared to the standard.
- 25 17. A method for detecting vascular disease in an individual comprising utilizing the method of claim 16 to determine the individual's Lp-PLA2 activity in a sample wherein increased activity of Lp-PLA2 in the sample is indicative of vascular disease.
18. The method of claim 17 wherein the vascular disease is selected from the group 30 consisting of coronary vascular disease (CVD), coronary heart disease (CHD), peripheral vascular disease, peripheral arterial disease, stroke, congenital cardiovascular defects and congestive heart failure.
19. A method for selecting an individual for therapy to treat vascular disease comprising utilizing the method of claim 16 to determine the individual's Lp-PLA2 activity in a sample wherein increased activity of Lp-PLA2 in the sample is indicative of an individual who will benefit from therapy to treat vascular disease.

20. The method of claim 19 wherein the vascular disease is selected from the group consisting of coronary vascular disease (CVD), coronary heart disease (CHD), peripheral vascular disease, peripheral arterial disease, stroke, congenital cardiovascular defects and congestive heart failure.
- 5 21. The method of claim 19 wherein the therapy is selected from the group consisting of statins and Lp-PLA2 inhibitors.
22. A method for monitoring an individual's response to therapy to treat vascular disease comprising utilizing the method of claim 16 to determine the individual's Lp-PLA2 activity in a sample wherein decreased activity of Lp-PLA2 in the sample is indicative of an individual who is responding favorably to therapy to treat vascular disease.
- 10 23. The method of claim 22 wherein the vascular disease is selected from the group consisting of coronary vascular disease (CVD), coronary heart disease (CHD), peripheral vascular disease, peripheral arterial disease, stroke, congenital cardiovascular defects and congestive heart failure.
- 15 24. The method of claim 22 wherein the therapy is selected from the group consisting of statins and Lp-PLA2 inhibitors.
25. A method for measuring enzymatically active Lipoprotein-associated Phospholipase A2 (Lp-PLA2) in a sample comprising:
  - 20 (a) contacting an binder, which specifically binds Lp-PLA2, with the sample to form a binder-Lp-PLA2 complex;
  - (b) immobilizing the binder-Lp-PLA2 complex;
  - (c) washing the immobilized binder-Lp-PLA2 complex to remove an enzymatically active unbound material or an interfering substance(s);
  - 25 (d) contacting the immobilized bound Lp-PLA2 with a substrate converted to a detectable product in the presence of Lp-PLA2; and
  - (e) measuring detectable product indicative of enzymatically active Lp-PLA2 in the sample.
- 30 26. The method of claim 25, wherein the sample is a serum sample, a plasma sample or an EDTA treated plasma sample.
27. The method of claim 25, wherein the binder is an antibody.
28. The method of claim 27, wherein the antibody is a monoclonal antibody, a phage display antibody, or a polyclonal antibody.

29. The method of claim 28, wherein the antibody is a monoclonal antibody.
30. The method of claim 25 wherein the binder-Lp-PLA2 complex is immobilized by binding to an immobilized compound.
31. The method of claim 30 wherein the immobilized compound is an antibody, protein or compound capable of binding the binder-Lp-PLA2 complex.
32. The method of claim 31, wherein the antibody is a monoclonal antibody, a phage display antibody, or a polyclonal antibody.
33. The method of claim 32, wherein the monoclonal antibody, the phage display antibody, or the polyclonal antibody is a rat, mouse or goat anti-Ig antibody.
34. The method of claim 30 wherein the immobilized compound is bound to a multi-well plate, a magnetic bead, or a latex bead.
35. The method of claim 25 wherein the binder is conjugated to an immobilizing agent.
36. The method of claim 35, wherein the binder conjugated to an immobilizing agent is an antibody.
37. The method of claim 36, wherein the antibody is a monoclonal antibody, a phage display antibody, or a polyclonal antibody.
38. The method of claim 37, wherein the antibody is a monoclonal antibody.
39. The method of claim 35 wherein the immobilizing agent is an antibody, protein or compound capable of binding an immobilized compound.
40. The method of claim 39, wherein the antibody is a monoclonal antibody, a phage display antibody, or a polyclonal antibody.
41. The method of claim 40, wherein the monoclonal antibody, the phage display antibody, or the polyclonal antibody is a rat, mouse or goat anti-Ig antibody.
42. The method of claim 35 wherein the immobilizing agent is biotin.
43. The method of claim 35 wherein the immobilizing agent, conjugated to the binder-Lp-PLA2 complex, binds to an immobilized compound.
44. The method of claim 43 wherein the immobilized compound is bound to a multi-well plate, a magnetic bead, or a latex bead.
45. The method of claim 44 wherein the bound compound is an antibody, protein or compound capable of binding the conjugated immobilizing agent.
46. The method of claim 45, wherein the antibody is a monoclonal antibody, a phage display antibody, or a polyclonal antibody.

47. The method of claim 46, wherein the monoclonal antibody, the phage display antibody, or the polyclonal antibody is a rat, mouse or goat anti-Ig antibody.

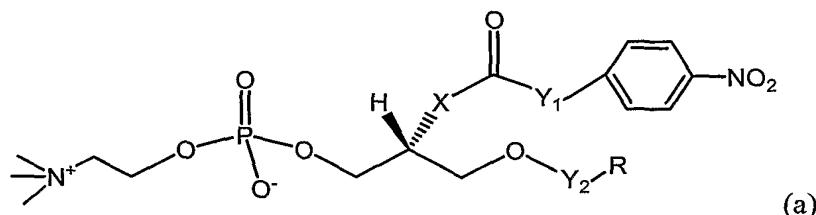
48. The method of claim 45 wherein the bound substance is streptavidin.

49. The method of claim 25 wherein the enzymatically active unbound material is a phospholipase.

50. The method of claim 25 wherein the interfering substance(s) is a free-thiol compound.

51. The method of claim 25, wherein the substrate is selected from the group consisting of

10



(a)

wherein,

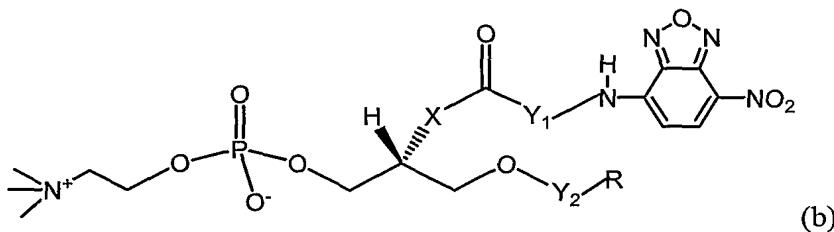
X is selected from the group consisting of O, S, and -O(CO)-;

R is selected from the group consisting of (CH<sub>2</sub>)<sub>4</sub>CH<sub>3</sub>, (CH<sub>2</sub>)<sub>6</sub>CH<sub>3</sub>, (CH<sub>2</sub>)<sub>8</sub>CH<sub>3</sub>, (CH<sub>2</sub>)<sub>10</sub>CH<sub>3</sub>, (CH<sub>2</sub>)<sub>12</sub>CH<sub>3</sub>, (CH<sub>2</sub>)<sub>14</sub>CH<sub>3</sub>, and (CH<sub>2</sub>)<sub>7</sub>CH=CH(CH<sub>2</sub>)<sub>2</sub>CH<sub>3</sub>;

Y<sub>1</sub> is selected from the group consisting of (CO)<sub>1-2</sub> and (CH<sub>2</sub>)<sub>2-7</sub>; and

Y<sub>2</sub> is selected from the group consisting of CO and CH<sub>2</sub>;

15



(b)

20

wherein,

X is selected from the group consisting of O, S, and -O(CO)-;

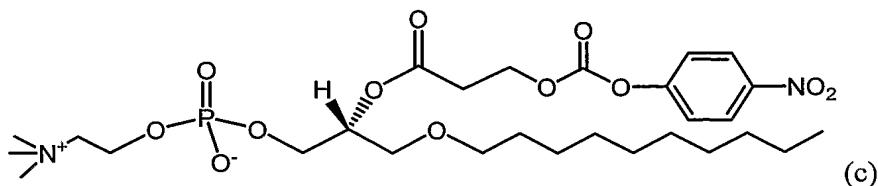
R is selected from the group consisting of (CH<sub>2</sub>)<sub>4</sub>CH<sub>3</sub>, (CH<sub>2</sub>)<sub>6</sub>CH<sub>3</sub>, (CH<sub>2</sub>)<sub>8</sub>CH<sub>3</sub>, (CH<sub>2</sub>)<sub>10</sub>CH<sub>3</sub>, (CH<sub>2</sub>)<sub>12</sub>CH<sub>3</sub>, (CH<sub>2</sub>)<sub>14</sub>CH<sub>3</sub> and (CH<sub>2</sub>)<sub>7</sub>CH=CH(CH<sub>2</sub>)<sub>2</sub>CH<sub>3</sub>;

Y<sub>1</sub> is selected from the group consisting of (CO)<sub>1-2</sub> and (CH<sub>2</sub>)<sub>2-7</sub>; and

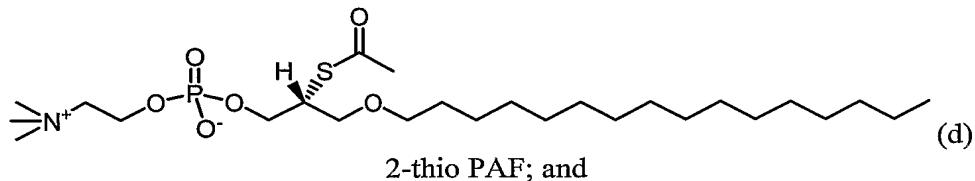
25

Y<sub>2</sub> is selected from the group consisting of CO and CH<sub>2</sub>;

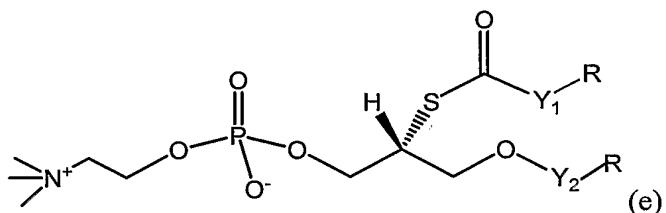
67



1-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine (MNP);



2-thio PAF; and



wherein

10 X is selected from the group consisting of O, S, and -O(CO)-;  
 R is selected from the group consisting of (CH<sub>2</sub>)<sub>4</sub>CH<sub>3</sub>, (CH<sub>2</sub>)<sub>6</sub>CH<sub>3</sub>, (CH<sub>2</sub>)<sub>8</sub>CH<sub>3</sub>, (CH<sub>2</sub>)<sub>10</sub>CH<sub>3</sub>,  
 (CH<sub>2</sub>)<sub>12</sub>CH<sub>3</sub>, (CH<sub>2</sub>)<sub>14</sub>CH<sub>3</sub>, and (CH<sub>2</sub>)<sub>7</sub>CH=CH(CH<sub>2</sub>)<sub>2</sub>CH<sub>3</sub>;  
 Y<sub>1</sub> is selected from the group consisting of (CO)<sub>1-2</sub> and (CH<sub>2</sub>)<sub>2-7</sub>; and  
 Y<sub>2</sub> is selected from the group consisting of CO or CH<sub>2</sub>.

15

- 52. The method of claim 51 where in the substrate is an oxidized derivative of (a), (b), (c), (d) or (e).
- 53. The method of claim 25, wherein the detectable product has a radioactive, colorimetric, paramagnetic or fluorescent label.

20

- 54. The method of claim 25 wherein the detectable product is measured fluorimetrically, colorimetrically, paramagnetically or via radiation.
- 55. The method of claim 25 further comprising comparing the measured detectable product of step (e) to detectable product in a control comprising an enzymatically active Lp-PLA2 standard.

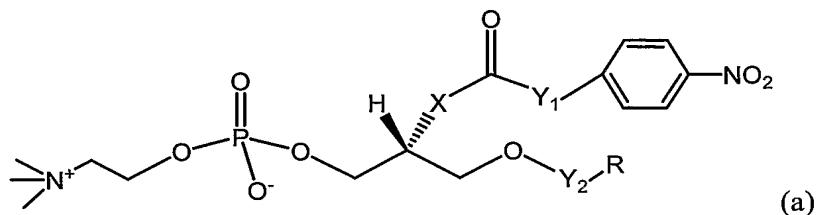
25

- 56. The method of claim 55 wherein the enzymatically active Lp-PLA2 standard is a recombinant Lp-PLA2 protein or a native Lp-PLA2 protein.

57. The method of claim 56 wherein the recombinant Lp-PLA2 protein is expressed in a baculovirus expression system or a mammalian expression system.
58. The method of claim 55 wherein a difference in detectable product in the sample compared to the standard is due to a difference in Lp-PLA2 activity in the sample compared to the standard.
59. A method for detecting vascular disease in an individual comprising utilizing the method of claim 58 to determine the individual's Lp-PLA2 activity in a sample wherein increased activity of Lp-PLA2 in the sample is indicative of vascular disease.
- 10 60. The method of claim 59 wherein the vascular disease is selected from the group consisting of coronary vascular disease (CVD), coronary heart disease (CHD), peripheral vascular disease, peripheral arterial disease, stroke, congenital cardiovascular defects and congestive heart failure.
- 15 61. A method for selecting an individual for therapy to treat vascular disease comprising utilizing the method of claim 58 to determine the individual's Lp-PLA2 activity in a sample wherein increased activity of Lp-PLA2 in the sample is indicative of an individual who will benefit from therapy to treat vascular disease.
- 20 62. The method of claim 61 wherein the vascular disease is selected from the group consisting of coronary vascular disease (CVD), coronary heart disease (CHD), peripheral vascular disease, peripheral arterial disease, stroke, congenital cardiovascular defects and congestive heart failure.
63. The method of claim 61 wherein the therapy is selected from the group consisting of statins and Lp-PLA2 inhibitors.
- 25 64. A method for monitoring an individual's response to therapy to treat vascular disease comprising utilizing the method of claim 58 to determine the individual's Lp-PLA2 activity in a sample wherein decreased activity of Lp-PLA2 in the sample is indicative of an individual who is responding favorably to therapy to treat vascular disease.
- 30 65. The method of claim 64 wherein the vascular disease is selected from the group consisting of coronary vascular disease (CVD), coronary heart disease (CHD), peripheral vascular disease, peripheral arterial disease, stroke, congenital cardiovascular defects and congestive heart failure.

5

- 66. The method of claim 64 wherein the therapy is selected from the group consisting of statins and Lp-PLA2 inhibitors.
- 67. A kit for measuring enzymatically active Lipoprotein-associated Phospholipase A2 (Lp-PLA2) in a sample comprising a binder which specifically binds Lp-PLA2 and a substrate converted to a detectable product in the presence of Lp-PLA2.
- 68. The kit of claim 67 wherein the substrate is selected from the group consisting of



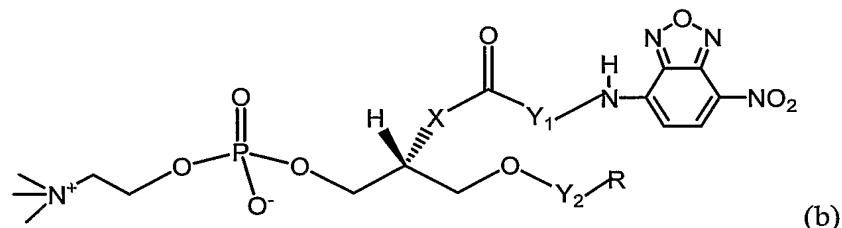
10 wherein,

X is selected from the group consisting of O, S, and -O(CO)-;

R is selected from the group consisting of  $(\text{CH}_2)_4\text{CH}_3$ ,  $(\text{CH}_2)_6\text{CH}_3$ ,  $(\text{CH}_2)_8\text{CH}_3$ ,  $(\text{CH}_2)_{10}\text{CH}_3$ ,  $(\text{CH}_2)_{12}\text{CH}_3$ ,  $(\text{CH}_2)_{14}\text{CH}_3$ , and  $(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_2\text{CH}_3$ ;

$Y_1$  is selected from the group consisting of  $(CO)_{1-2}$  and  $(CH_2)_{2-7}$ ; and

15 Y<sub>2</sub> is selected from the group consisting of CO and CH<sub>2</sub>;



wherein,

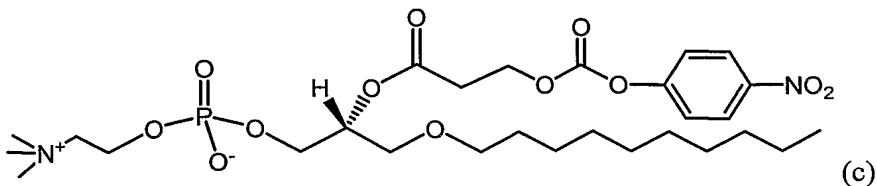
X is selected from the group consisting of O, S, and -O(CO)-;

20 R is selected from the group consisting of  $(\text{CH}_2)_4\text{CH}_3$ ,  $(\text{CH}_2)_6\text{CH}_3$ ,  $(\text{CH}_2)_8\text{CH}_3$ ,  $(\text{CH}_2)_{10}\text{CH}_3$ ,  $(\text{CH}_2)_{12}\text{CH}_3$ ,  $(\text{CH}_2)_{14}\text{CH}_3$  and  $(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_2\text{CH}_3$ ;

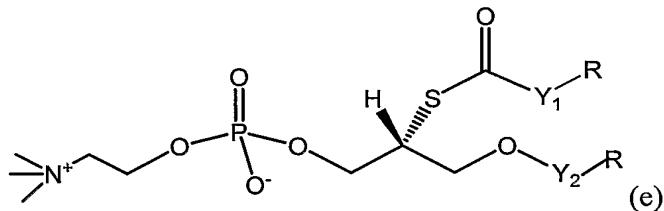
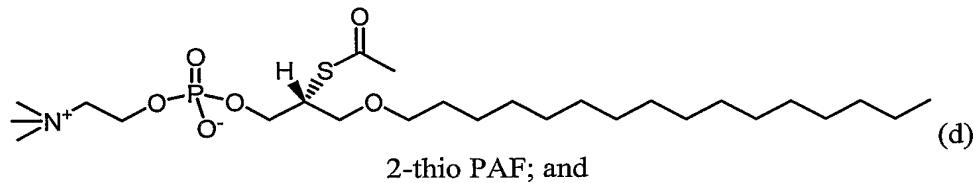
$Y_1$  is selected from the group consisting of  $(CO)_{1-2}$  and  $(CH_2)_{2-7}$ ; and

$Y_2$  is selected from the group consisting of CO and CH<sub>2</sub>;

70



1-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine (MNP);



wherein

10 X is selected from the group consisting of O, S, and -O(CO)-;

R is selected from the group consisting of  $(\text{CH}_2)_4\text{CH}_3$ ,  $(\text{CH}_2)_6\text{CH}_3$ ,  $(\text{CH}_2)_8\text{CH}_3$ ,  $(\text{CH}_2)_{10}\text{CH}_3$ ,  $(\text{CH}_2)_{12}\text{CH}_3$ ,  $(\text{CH}_2)_{14}\text{CH}_3$ , and  $(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_2\text{CH}_3$ ;

$\text{Y}_1$  is selected from the group consisting of  $(\text{CO})_{1-2}$  and  $(\text{CH}_2)_{2-7}$ ; and

$\text{Y}_2$  is selected from the group consisting of CO or  $\text{CH}_2$ .

15

69. The kit of claim 68 wherein the substrate is an oxidized derivative of (a), (b), (c), (d) or (e).
70. The kit of claim 67 further comprising an enzymatically active Lp-PLA2 standard.

20

71. The kit of claim 70 wherein the enzymatically active Lp-PLA2 standard is a recombinant Lp-PLA2 protein or a native Lp-PLA2 protein.
72. The kit of claim 71 wherein the recombinant Lp-PLA2 protein is expressed in a baculovirus expression system or a mammalian expression system.
73. A method for measuring enzymatically active Lipoprotein-associated Phospholipase A2 (Lp-PLA2) in a sample comprising:

25

(a) incubating the sample with a compound which reduces active thiol(s) in the sample;

(b) contacting the incubated sample with a substrate converted to a free thiol product in the presence of enzymatically active Lp-PLA2; and

5 (c) measuring free thiol product indicative of enzymatically active Lp-PLA2 in the sample.

74. The method of claim 73, wherein the sample is a serum sample, a plasma sample or an EDTA treated plasma sample.

10 75. The method of claim 73 wherein the compound which reduces active thiol(s) in the sample is DTNB.

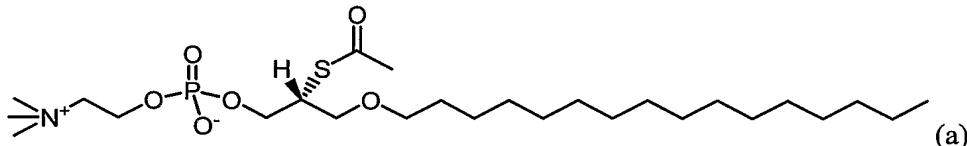
76. The method of claim 73 wherein the sample is incubated at room temperature.

77. The method of claim 73 wherein the sample is incubated at 37°C.

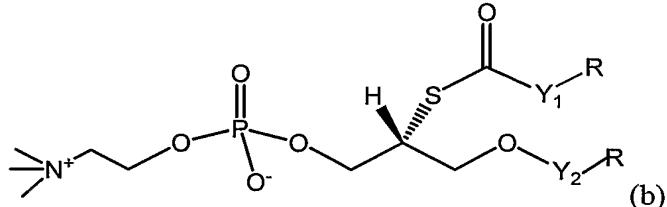
78. The method of claim 73 wherein the sample is incubated from about 2 to about 120 minutes.

15 79. The method of claim 73 wherein the sample is incubated from about 5 to about 30 minutes.

80. The method of claim 73 wherein the substrate is selected from the group consisting of



20 2-thio PAF; and



25 wherein,

R is selected from the group consisting of  $(\text{CH}_2)_4\text{CH}_3$ ,  $(\text{CH}_2)_6\text{CH}_3$ ,  $(\text{CH}_2)_8\text{CH}_3$ ,  $(\text{CH}_2)_{10}\text{CH}_3$ ,  $(\text{CH}_2)_{12}\text{CH}_3$ ,  $(\text{CH}_2)_{14}\text{CH}_3$  and  $(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_2\text{CH}_3$ ;

$\text{Y}_1$  is selected from the group consisting of  $(\text{CO})_{1-2}$  and  $(\text{CH}_2)_{2-7}$ ; and

$\text{Y}_2$  is selected from the group consisting of CO and CH<sub>2</sub>.

81. The method of claim 80 where in the substrate is an oxidized derivative of (a) or (b).

82. The method of claim 73 further comprising comparing measured free thiol product of step (c) to free thiol product in a control comprising an enzymatically active Lp-PLA2 standard.

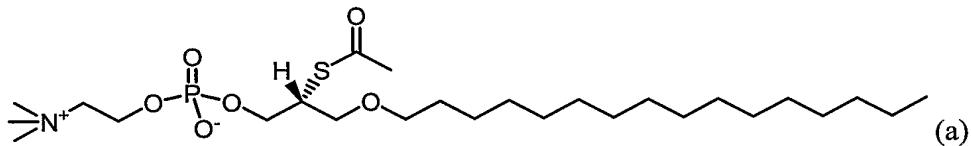
5       83. The method of claim 82 wherein the enzymatically active Lp-PLA2 standard is a recombinant Lp-PLA2 protein or a native Lp-PLA2 protein.

84. The method of claim 83 wherein the recombinant Lp-PLA2 protein is expressed in a baculovirus expression system or a mammalian expression system.

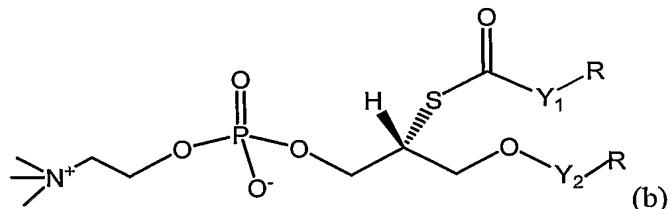
10      85. The method of claim 73 wherein the steps (a), (b), and (c) are conducted in a multi-well plate.

86. A kit for measuring enzymatically active Lipoprotein-associated Phospholipase A2 (Lp-PLA2) in a sample comprising a compound which reduces active thiol(s) and a substrate converted to a detectable product in the presence of Lp-PLA2.

15      87. The kit of claim 86 wherein the substrate is selected from the group consisting of



2-thio PAF; and



wherein,

R is selected from the group consisting of  $(CH_2)_{4}CH_3$ ,  $(CH_2)_{6}CH_3$ ,  $(CH_2)_{8}CH_3$ ,  $(CH_2)_{10}CH_3$ ,  
25       $(CH_2)_{12}CH_3$ ,  $(CH_2)_{14}CH_3$  and  $(CH_2)_7CH=CH(CH_2)_2CH_3$ ;

$Y_1$  is selected from the group consisting of  $(CO)_{1-2}$  and  $(CH_2)_{2-7}$ ; and

$Y_2$  is selected from the group consisting of CO and  $CH_2$ .

88. The kit of claim 87 where in the substrate is an oxidized derivative of (a) or (b).
89. The kit of claim 86 further comprising an enzymatically active Lp-PLA2 standard.
- 5 90. The kit of claim 89 wherein the enzymatically active Lp-PLA2 standard is a recombinant Lp-PLA2 protein or a native Lp-PLA2 protein.
91. The kit of claim 90 wherein the recombinant Lp-PLA2 protein is expressed in a baculovirus expression system or a mammalian expression system.